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Acclimation capacity of the cardiac HSP70 and HSP90 response to thermal stress in lake trout (*Salvelinus namaycush*), a stenothermal ice-age relict

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ABSTRACT

The ability of cold-adapted species to persist in the face of climate change will depend on the capacity of individuals for thermal acclimation, adaptation, and the degree of physiological variation that exists among populations. We tested the acclimation capacity of cardiac HSP70 and HSP90 in four populations of lake trout (*Salvelinus namaycush*), a cold-adapted salmonid. We acclimated fish to four temperatures (8 °C, 11 °C, 15 °C, and 19 °C) and measured cardiac HSP70 and HSP90 levels by western blot prior to and following an acute (1 h) heat shock at 23 °C. Basal and induced cardiac HSP70 and HSP90 expression was similar among lake trout populations. Induced cardiac HSP70 was significantly lower in heat-shocked lake trout acclimated to 19 °C compared to all other temperatures. Acclimation temperature had no significant effect on cardiac HSP90 prior to heat shock (control HSP90) and a significant effect on HSP90 in heat-shocked fish, although most significant treatment comparisons were marginal. However, for warm-acclimated fish (15 °C and 19 °C), cardiac HSP90 levels were lower in heat-shocked versus control fish. Together, this suggests that the induction temperature of cardiac HSP synthesis may increase with warm acclimation in lake trout. This plasticity in the cardiac HSR could assist lake trout populations in coping with longer periods of thermal stress as predicted by climate change models. The cardiac HSR supports previous research suggesting lake trout thermal physiology may be conserved across a wide geographic range.

1. Introduction

Climate change is predicted to have significant impacts on freshwater ecosystems and their resident biota (Ficke et al., 2007; Shuter et al., 2012; Lynch et al., 2016). Over the next century, average near-surface air temperature and thermal heterogeneity are projected to increase, particularly at higher latitudes (IPCC, 2014). Elevated water temperatures will directly affect a variety of physiological processes in freshwater fishes (Whitney et al., 2016), with implications for the geographical distributions of species (Chu et al., 2005), phenological processes (Shuter et al., 2012) and trophic interactions (Yang and Rudolf, 2010).

Cold-adapted freshwater species may be particularly vulnerable to climate change (Meisner, 1990; Chu et al., 2005; Gerdeaux, 2011). A chronic increase in air temperature is expected to alter the thermal properties of lacustrine habitats and increase the magnitude and duration of thermal stratification resulting in a spatial and temporal decline in optimal thermal habitat for coldwater species (De Stasio et al., 1996; Stefan et al., 1998; Ficke et al., 2007). Empirical studies have already documented climate-related declines in abundance (Jacobsen et al., 2012), growth (McDonald et al., 1996; Murdoch and Power, 2013), recruitment, and population size (Bassar et al., 2016) for North American coldwater fish species. Cold-adapted populations unable to shift their geographical distribution may face extirpation across much of their current range (Casselman, 2002; Chu et al., 2005), and their persistence will likely depend on their capacity for physiological adaptation or acclimatization to elevated temperatures (Somero, 2010). The capacity to acclimatize to new thermal environments will determine whether populations are able to persist long enough for evolutionary adaptation to occur (Stillman, 2003; Somero, 2010). However, this capacity may be limited in some species due to limited genetic variation from small ancestral populations in glacial refugia and post-colonization constraints to local population sizes (Bernatchez and Wilson, 1998; Willi et al., 2006; Wells et al., 2016). To accurately

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predict how cold-adapted species will respond to climate change, we must understand the degree of variation that exists among populations for relevant physiological traits, and the plasticity of these traits under different thermal conditions (Seebacher et al., 2015).

One physiological mechanism that plays a significant role in whole-organism thermal tolerance is the heat shock response (HSR; Feder and Hofmann, 1999). The HSR produces a family of heat shock proteins (HSPs) that function as molecular chaperones to prevent protein aggregation and denaturation, and to maintain protein homeostasis during periods of thermal stress (Iwama et al., 1999). Two highly conserved proteins from the HSR are heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90) (Lindquist and Craig, 1988). HSP70 functions as a molecular chaperone to assist with the folding of nascent polypeptide chains and mediate the repair and degradation of denatured proteins. HSP90 plays a variety of roles in supporting the cytoskeleton, enzymes and steroid hormone receptors (Iwama et al., 2004). The expression of HSPs in response to a thermal challenge has been documented for a variety of fish species (Dietz and Somero, 1992; Fangue et al., 2006), including salmonids (Narum et al., 2013; Stitt et al., 2014; Corey et al., 2017). In addition, HSPs have ecological relevance. For example, the temperature of HSP70 induction in brook trout (*Salvelinus fontinalis*) correlates with the thermal ecological limits of this species in the wild (Chadwick Jr. et al., 2015), and levels of HSP70 expression covary with differences in thermal tolerance among populations (Stitt et al., 2014). Variation among populations in the magnitude of the HSR, and the capacity for acclimation, may play a significant role in determining the ecological response of species to climate change (Tomanek, 2008, 2010; Somero, 2010).

The lake trout (*Salvelinus namaycush*) is a cold-adapted salmonid that evolved during the Pleistocene glaciations (Martin and Olver, 1980; Wilson and Hebert, 1996, 1998) and is an excellent model in which to investigate mechanisms underlying thermal tolerance and physiological acclimation in coldwater fishes. During the summer months when lake temperatures are thermally stratified, lake trout migrate to deep coldwater habitat and generally occupy temperatures within their optimum range (10 °C ± 2 °C; Plumb and Blanchfield, 2009) aside from brief forays into shallow warmwater habitat to forage (Morbey et al., 2006) or to avoid limited oxygen conditions at depth (Myrt Lake; J. McDermid, DFO, personal communication). Climate change is predicted to significantly impact lake trout populations through a decline in suitable summer thermal habitat, an increase in exposure to suboptimal temperatures and thermal stress (Ficke et al., 2007; Guzzo and Blanchfield, 2016) and a reduction in growth and condition (Plumb et al., 2014; Guzzo et al., 2017). Assessing their ability to physiologically cope with higher temperatures, the degree of variation among lake trout populations in their response to thermal stress, and their capacity for thermal acclimation is driven by climate change concerns and relevant to their long-term sustainability.

Previously, we showed that lake trout from four populations in Ontario can, to a limited extent, increase their thermal tolerance via acclimation. However, we detected little variation among individual populations in acclimation capacity or in whole-organism physiological traits (Kelly et al., 2014). This study included two well-studied populations of the two most common inland lake ecotypes as representatives (Louisa and Opeongo), as well as the stocking source most commonly used for stocking inland lakes in Ontario (Lake Manitou), and a thermally-stressed population from northwestern Ontario (Myrt Lake) where lake trout are limited to the metalimnion due to low oxygen in the hypolimnion (Myrt Lake is amictic) and high temperatures in the epilimnion (J. McDermid, DFO, unpubl. data). We hypothesized that HSP expression may underlie these whole-organism patterns and thus, we tested the roles that thermal acclimation and population of origin would have on cardiac HSP70 and HSP90 expression in lake trout exposed to a sub-lethal heat shock. We acclimated juvenile lake trout from the same four populations, reared from eggs in a common environment, to four temperatures (8 °C, 11 °C, 15 °C and 19 °C) and sampled fish at each acclimation temperature (basal) and after being heat shocked to 23 °C and allowed to recover for 24 h. We predicted that cardiac HSP70 and HSP90 levels would be subject to thermal acclimation (i.e. distinct induction profiles at each acclimation temperature), and that this relationship would be similar among populations of juvenile lake trout. We focused on the heart because this tissue may be particularly vulnerable to thermal stress in juvenile salmonids. The magnitude of the HSR is significantly greater in the hearts of juvenile rainbow trout (*Oncorhynchus mykiss* (Fowler et al., 2009). In addition, juvenile chinook salmon (*Oncorhynchus tshawytscha*) exposed to a 10.6 °C heat shock for 5 h displayed a larger increase in HSP90 mRNA in cardiac tissue compared to all other tissues examined (brain, gill, muscle, liver, kidney and tail fin; Palmisano et al., 2000). Cardiac function plays an important role in limiting upper thermal tolerance and aerobic scope in fishes, particularly salmonids (Farrell et al., 2009; Eliason et al., 2011). Furthermore, previous research has demonstrated remarkable plasticity (Anttila et al., 2014a) and local adaptation (Eliason et al., 2011; Chen et al., 2013; Gradil et al., 2016) of salmonid cardiac physiology to environmental temperature, including the HSR (Anttila et al., 2014b).

2. Materials and methods

These studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Institutional Animal Care Committee of Trent University (Protocol # 22261) and the Ontario Ministry of Natural Resources and Forestry Aquatic Animal Care committee (Protocol # 92).

2.1. Lake trout populations

The lake trout used in this study were from four populations in Ontario, Canada that were chosen based on a combination of scientific research and fisheries management purposes. Two populations (Opeongo Lake and Lake Louisa) were established one generation ago from wild spawn collections from these lakes in Algonquin Provincial Park (southcentral Ontario; 45°47′N, 78°12′W). These populations are native (Wilson and Hebert, 1996, 1998; Halbisen and Wilson, 2009), and previous research suggests that the thermal sensitivity of growth and thermal tolerance of these two populations may differ from each other (McDermid et al., 2013; but see Kelly et al., 2014). The Myrt Lake population was established from wild spawn collections from Myrt Lake in northwestern Ontario (48°26′N, 90°43”W), a small (273 ha) lake where lake trout are forced to occupy suboptimal temperatures (21 °C) at shallow depths to avoid limiting oxygen conditions in deeper coldwater habitat (J. McDermid, personal communication). Lake trout in Myrt Lake are also presumed to be native, as no stocking records exist for this lake (Ontario Ministry of Natural Resources and Forestry (OMNRF) stocking database). The Lake Manitou population was established from Lake Manitou on Manitoulin Island (45°45′N, 81°57”W) and has been used as a source for fish stocking for over 50 years, using fresh wild spawn collections once per generation (OMNR, 2005). Understanding the physiological capacity for thermal acclimation in this stocking source as well as how these fish compare to other populations is important for fisheries management. All of the source populations used for this experiment have been reciprocally isolated for at least 1000 generations (Wilson and Hebert, 1996, 1998; Wilson and Mandrak, 2004). The two source populations in Algonquin Park have no direct water connections, and have not since their initial colonization during the retreat of the Laurentide Ice Sheet. Similarly, lake trout in Lake Manitou on Manitoulin Island in Lake Huron were trapped by receding waters of Glacial Lake Algonquin approximately 10,000 years ago. The population in Myrt Lake in northwestern Ontario had a separate colonization history, although is also descended from an ancestral Mississippian refugial population at the end of the Pleistocene (C. Wilson, OMNRF, unpubl. data). Due to logistical limitations on transporting fish or gametes across jurisdictional (provincial / state /

federal) borders, it was not possible to assess variation among populations across the entire species' range, with both geographic and phylogenetic representation.

In the fall of 2009, adults within each population were spawned using single-pair monogamous crosses (mating one female with one male) to make four families per population at the OMNRF Codrington Fisheries Research Facility (Codrington, Ontario). This flow-through facility is supplied by spring-fed surface headwaters of Marsh Creek, impounded in a controlled-depth head pond; yearly temperatures fluctuate seasonally between 2 °C and 14 °C (OMNRF, unpubl. data). Eggs were reared in family-specific lots in identical partitioned Heath trays until hatching. Once hatched, each family of sac fry was transferred to its own 20 L flow-through tank and maintained separately from other families. Although families were held in separate tanks, fish in all 16 tanks experienced the same controlled environmental and feeding conditions through all life stages prior to our thermal acclimation experiment. All fish used in this study were one generation removed from the wild. For each population, the number of fish per family was balanced. Throughout the rearing process, the number of fish per family was kept relatively constant at each life stage to ensure equal representation of families and minimize effects from hatchery selection. Since all families in each population were equally represented and only one generation removed from the wild, there has been little opportunity for transgenerational adaptation within any of the populations.

In October 2010, families from the different populations were put into 200 L oval acclimation tanks (Frigid Units Inc., Toledo, OH, USA), with two tanks per population. Each family (N = 150) were evenly split between the two tanks for a given populations, so that each tank held a total of 300 fish (75 fish·family^{-1}·4 families·tank^{-1}). From that point on, it was not possible to identify fish back to family, only population. In November 2011, two-year old lake trout from all populations received population-specific visible implant elastomer tags (VIE; Northwest Marine Technologies, Shaw Island, WA, USA). Directly after tagging, fish were randomly and evenly divided (38 fish·population^{-1}·tank^{-1}; n = 1216) among eight 200 L thermal acclimation tanks at ambient water temperature (approximately 8 °C). Together, this ensured that fish from all four populations were held under identical conditions for each temperature but remained distinguishable based on VIE markings.

2.2. Experimental design

Tanks were randomly assigned to one of four target temperatures (8 °C, 11 °C, 15 °C and 19 °C), with two acclimation tanks assigned to each treatment (76 individuals·population^{-1}·acclimation temperature^{-1}). These acclimation temperatures were chosen based on previous research conducted on lake trout; juvenile lake trout typically occupy 8 °C in the summer hypolimnion (Plumb and Blanchfield, 2009), 11 °C is within the optimal temperature range for growth (O'Connor et al., 1981; Christie and Regier, 1988) and the preferred temperature range for lake trout (McCauley and Tait, 1970; McDermid et al., 2013). Aerobic metabolic scope is maximized at approximately 15 °C, and although 19 °C is warmer than the thermal optimum for lake trout, it is below the incipient lethal temperature (Evans, 2007; Kelly et al., 2014).

In May and June of 2012, ~2.5 year-old fish were acclimated to the four target temperatures (8 °C, 11 °C, 15 °C and 19 °C), from ambient water temperature, at a rate of 1 °C·day^{-1} using programmable heaters (Finnex TH-0300 and TH-0500, IL, USA). Once acclimation temperatures had been achieved, they were maintained ± 1.0 °C for a minimum of four weeks before experiments began. Water quality was maintained using a partial recirculation system (80% recirculation) through water filtration pumps (Eheim; Dollard Des Ormeaux, QC, Canada), which were cleaned weekly. Compressed air was supplied to each tank using 58 cm air stones to ensure adequate water circulation, temperature homogeneity and oxygen supply (> mg·L^{-1}). Water quality parameters were monitored at least once per week for nitrate, nitrite, ammonia and pH levels. Water temperature was recorded in each tank with Tidbit V2 Water Temperature loggers (Onset HOBO data loggers, Pocasset, MA, USA). The average water temperature of each acclimation tank was calculated for the entire period of thermal acclimation prior to starting heat shock experiments (Table 1). Fish were fed to satiation twice daily using 3.0 mm Optimum Salmonid feed (COREY Nutrition Co., Fredericton, NB; Kelly et al., 2014).

Table 1
Desired and achieved acclimation temperatures for eight acclimation tanks prior to heat shock experiments. Values are means SEM for each acclimation temperature and tank.

Desired acclimation temperature (°C)	Acclimation tank	Mean temperature before heat shock experiments (°C)
8	1	8.75 ± 0.013
	2	8.75 ± 0.013
11	3	11.02 ± 0.008
	4	10.87 ± 0.008
15	5	15.01 ± 0.004
	6	15.23 ± 0.005
19	7	18.68 ± 0.007
	8	19.29 ± 0.003

2.3. Heat shock challenge

Groups of fish from each of the 4 populations (5 individuals·population^{-1}·trial^{-1}; 2 heat shock trials·acclimation·temperature^{-1}; n = 160) were introduced into one of two 172 L thermal challenge tanks (n = 20 per trial) at their acclimation temperature, and allowed to acclimate to the tank overnight. Both experimental tanks received continuous aeration and were monitored using a dissolved oxygen probe (YSI Pro 20; ± 0.2 mg·L^{-1}) to maintain a dissolved oxygen concentration above 6 mg·L^{-1}. Acclimation temperatures were maintained (± 0.5 °C) overnight using 500 W heaters (Finnex TH-0300 and TH-0500, IL, USA). Fish were not fed for 24 h prior to experimental trials.

After overnight acclimation, water temperature was increased by 3 °C·h^{-1} from the acclimation temperature to a maximum of 23 °C. This heat shock temperature was chosen based on prior upper thermal tolerance experiments, as it was below the critical thermal maximum (CTM) for juvenile lake trout (approximately 26 °C – 29 °C across the acclimation temperature range used in this study; Kelly et al., 2014). Temperature was controlled by balancing the inflow of ambient water (ca. 7 °C for the 8 °C and 11 °C acclimation group; 13 °C for the 15 °C and 19 °C acclimation groups), and the flow of hot water (ca. 60 °C) through four aluminum-plated heating elements at the base of the tank. Fish were held at 23 °C for 1 h, after which water temperature was cooled to the initial acclimation temperature over 1 h. Fish were allowed to recover for 24 h, and were then euthanized via a lethal blow to the head. Multiple studies that have investigated the HSR in juvenile salmonids have demonstrated that this length of a recovery period is sufficient for measuring maximum cardiac HSP expression (Rendell et al., 2006; Fowler et al., 2009; Gallant et al., 2017). Measurements of length and weight were made, whole cardiac tissue was rapidly removed, immediately frozen in liquid nitrogen and then stored at − 80 °C. To quantify HSP levels prior to the heat shock challenge, fish from each population (10 individuals·population^{-1}·acclimation temperature^{-1}; 160 individuals total) were removed from their respective acclimation tanks, immediately euthanized, weighed, measured and dissected as above. All experiments were conducted within a two-week period.

2.4. Heat shock protein quantification

Soluble protein was extracted from cardiac tissue using methods described in LeBlanc et al. (2011). Briefly, whole cardiac tissue was ground under liquid nitrogen, homogenized, pushed through a 27-ga needle three times to shear DNA and centrifuged at 14000*g* for 10 min. The supernatant was carefully removed and transferred into a new vial. Soluble protein concentrations were quantified for each sample using a Bio−Rad (Hercules, CA) DC protein assay, with bovine serum albumin as a standard. Microplates were read at 750 nm using a 96-well tunable microplate reader (Versa Max, Molecular Devices, Sunnyvale, CA). HSP70 and HSP90 from cardiac tissues (15 μg of soluble protein) were quantified through Western blotting using a Novex XCell gel system (Invitrogen, Carlsbad CA). For both HSP70 and HSP90, a sample from a heat-shocked fish was loaded onto every gel to act as an internal control and to allow direct comparison between gels. Rabbit anti-salmonid-inducible HSP70 and HSP90 polyclonal antibodies (Agrisera, Vannas, SE, Product Numbers AS09 061 and AS05 063, respectively) were used for immunodetection. The HSP70 antibody only recognizes the inducible isoform of HSP70 and not the constitutive isoform. The HSP90 antibody we used was not designed to specifically distinguish between constitutive and inducible isoforms, therefore, we took the conservative approach of quantifying HSP90 isoforms together rather than attempting to separate isoforms by running the gels for longer. Goat anti-rabbit IgG (Agrisera, Vannas, SE, Product Number AS09 602) was used as the secondary antibody and detected using ECL Advance Chemiluminescent Western Blotting Detection Kit (GE Healthcare, Baie d'Urfe, QC). Protein bands were quantified using a VersaDoc MP 45000 (Bio−Rad) molecular imager and Image Lab software (version 3.0; Bio−Rad). The relative band density of HSP70 and HSP90 was calculated for each fish by dividing the sample band density by the band density of the control sample loaded on every gel.

2.5. Statistical analyses

We tested for differences in fork length and body mass among populations and acclimation temperatures using a two-way analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test for post-hoc pairwise comparisons. To analyze HSP70 and HSP90 levels both prior to heat shock (basal HSP at each acclimation temperature) and 24 h after an acute heat shock to 23 °C (induced HSP), we originally performed separate two-way analysis of variance (ANOVA), with acclimation temperature and population of origin as main effects, and their interaction. However, graphical exploration of model residuals revealed significant heteroscedasticity, mainly driven by the acclimation temperature factor. Data transformations did not remedy the situation; thus, a two-factor ANOVA was not suitable because of this violation of a fundamental model assumption. We instead incorporated this heteroscedasticity directly into our analysis using Generalized Least Squares (GLS) models, and allowed HSP70 and HSP90 variances to differ among acclimation temperature treatments. This statistical modeling technique does not require variance homogeneity and is a recommended approach when analyzing heteroscedastic data (Zuur et al., 2010). All statistical tests on HSP data were performed using the nlme package (Pinheiro et al., 2017) in R (version 3.3.2 for PC) with $p < 0.05$ as the level of significance. Interaction terms were always retained in the GLS model. In the event of a significant main effect (significant effect of acclimation temperature or population of origin), Dunn's test was used followed by a Bonferroni correction to adjust for multiple comparisons to determine which groups differed from each other. In addition, because the HSP90 antibody does not discriminate between HSP90 isoforms, we calculated the difference in mean HSP90 of heat-shocked fish and basal fish at acclimation temperature to better assess the effect of heat shock on cardiac HSP90 expression (see Fig. 3). These calculations were made for all populations at all acclimation temperatures using least squares means from the GLS model outputs. All figures were produced using the ggplot2 package (Wickham, 2009) in R (version 3.3.2 for PC).

The original experimental design was established to measure basal (non-heat shocked) and induced (heat-shocked) cardiac HSP70 and HSP90 in four lake trout populations across four acclimation temperatures (10 fish·populations^{-1}·acclimation temperature^{-1}·heat shock treatment^{-1}). However, after further inspection of the basal cardiac HSP90 western blots we discovered a problem at the enhanced chemiluminescence detection stage of the immunoblotting for samples from all fish acclimated to 8 °C and half of the fish acclimated to 11 °C. Poor-quality internal standards resulted in these blots being unquantifiable. Therefore, we decided to omitthese data and analyze basal cardiac HSP90 data from the remaining data set of 5 fish·population^{-1}·acclimation temperature^{-1} for the 11 °C acclimation temperature treatment, and 10 fish·population^{-1}·acclimation temperature^{-1} for the 15 °C and 19 °C treatments. The basal HSP90 results presented in text and figures below are based on this subset of data.

3. Results

3.1. Physical characteristics of Lake trout populations

There was no significant difference in body mass among populations or acclimation temperatures (Table 2; $F_{3,304} = 1.43$, $p = 0.24$ and $F_{3,304} = 1.68$, $p = 0.17$, respectively). In contrast, fork length varied significantly among both acclimation temperatures ($F_{3,304} = 7.56$, $p < 0.001$) and populations ($F_{3,304} = 3.09$, $p < 0.05$). Juvenile lake trout acclimated to 19 °C were significantly shorter than fish acclimated to 8 °C, 11 °C and 15 °C (Tukey's HSD; $p < 0.001$ for all comparisons). Lake Manitou fish were significantly longer than lake trout from Opeongo Lake (Tukey's HSD; $p < 0.05$). There was no significant interaction between acclimation temperature and population for body mass or fork length (Table 2; $F_{9,304} = 0.94$, $p = 0.49$ and $F_{9,304} = 0.55$, $p = 0.84$, respectively).

3.2. HSP70

HSP70 was not detected in whole cardiac muscle prior to heat shock (data not shown). Twenty-four hours following a 1 h acute heat shock, HSP70 was induced, and levels in cardiac tissue varied significantly among acclimation temperatures (Fig. 1; $F_{3,144} = 81.84$, $p < 0.001$). Following heat shock, inducible cardiac HSP70 was significantly higher in 11 °C-acclimated fish compared to fish acclimated to 15 °C (Dunn's test with Bonferroni correction: $p < 0.05$; Fig. 1). In addition, induced

Table 2
Body mass (g) and fork length (cm) of four lake trout populations at four acclimation temperatures at the start of acute heat shock experiments, showing sample sizes measured (N) and means ± standard errors.

Acclimation Temperature (°C)	Population	N	Mass (g)	Fork Length (cm)
8	Myrt Lake	20	65.0 ± 3.1	174.4 ± 2.5
	Lake Louisa	20	65.6 ± 3.6	177.4 ± 3.3
	Opeongo Lake	20	57.9 ± 4.4	172.1 ± 3.6
	Lake Manitou	20	62.7 ± 3.3	178.0 ± 2.7
11	Myrt Lake	20	66.4 ± 2.9	175.8 ± 2.2
	Lake Louisa	20	61.1 ± 4.1	172.2 ± 3.5
	Opeongo Lake	20	61.4 ± 2.8	174.7 ± 2.4
	Lake Manitou	20	62.9 ± 3.2	177.7 ± 3.5
15	Myrt Lake	20	66.2 ± 4.0	172.5 ± 3.3
	Lake Louisa	20	67.4 ± 5.6	174.6 ± 5.0
	Opeongo Lake	20	58.5 ± 3.6	169.2 ± 3.0
	Lake Manitou	20	64.5 ± 4.4	177.9 ± 4.1
19	Myrt Lake	20	61.7 ± 4.2	166.1 ± 2.8
	Lake Louisa	20	49.9 ± 3.9	160.8 ± 3.4
	Opeongo Lake	20	59.8 ± 4.8	162.9 ± 3.8
	Lake Manitou	20	61.8 ± 4.0	172.8 ± 3.3

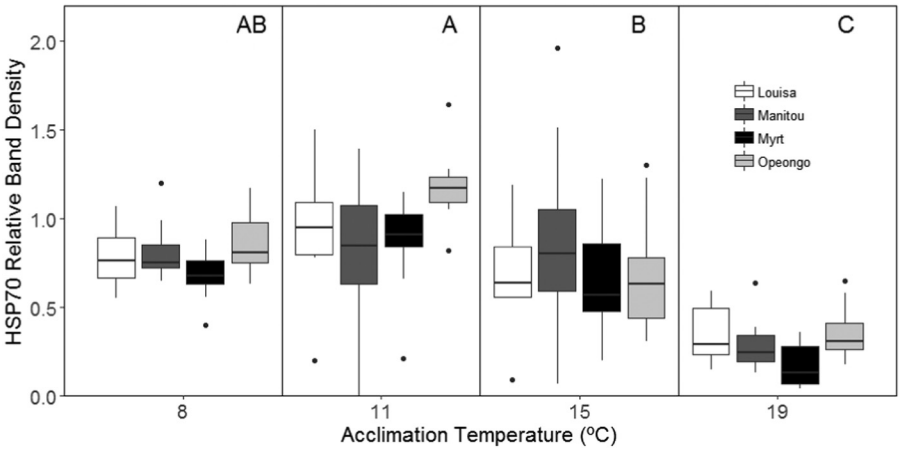

Fig. 1. Inducible HSP70 response of four lake trout populations acclimated to four temperatures [8 °C, 11 °C, 15 °C, and 19 °C] following acute heat shock (1 h) to 23 °C. Levels are expressed relative to a control (heat-shocked sample). The boxes represent the first and third quartiles, the horizontal lines represent the median, the whiskers represent fifth and ninety-fifth percentiles, and points outside of these whiskers represent outliers. All outliers were retained in the analysis. Significant differences among acclimation temperatures based on Dunn's test with Bonferroni correction for multiple comparisons ($p < 0.05$) are indicated by upper case letters. Significant differences among populations based on Dunn's test with Bonferroni correction for multiple comparisons ($p < 0.05$) are indicated by lowercase letters in legend. Acclimation and population had a significant effect on cardiac HSP70 in heat-shocked fish ($p < 0.001$).

cardiac HSP70 was significantly lower in 19 °C-acclimated fish when compared with fish at all other acclimation temperatures (Dunn's test with Bonferroni correction: $p < 0.05$ for all comparisons). Inducible HSP70 also varied among populations ($F_{3,144} = 5.42$, $p < 0.01$); however, post hoc pairwise comparisons detected no significant difference between any of the populations (Dunn's test with Bonferroni correction; $p > 0.05$ for all comparisons). There was no significant interaction between acclimation temperature and population (Fig. 1; $F_{9,144} = 0.74$, $p = 0.67$), indicating that all populations responded to the heat shock in the same way, irrespective of acclimation temperature.

3.3. HSP90

Prior to heat shock, basal levels of HSP90 were detected in cardiac tissue in all populations and at all acclimation temperatures (Fig. 2a). There was no significant effect of acclimation temperature (Fig. 2a; $F_{2,88} = 2.75$, $p = 0.07$) or population ($F_{3,88} = 0.04$, $p = 0.99$) on basal cardiac HSP90 expression. There was no significant interaction between acclimation temperature and population for basal HSP90 expression (Fig. 2(a); $F_{6,88} = 0.47$, $p = 0.83$).

Following an acute heat shock, cardiac HSP90 varied significantly with acclimation temperature (Fig. 2(b) $F_{3,144} = 29.51$, $p < 0.001$). Lake trout acclimated to 11 °C had significantly higher HSP90 levels compared with all other acclimation temperatures (Dunn's test with Bonferroni correction: $p < 0.01$ for all comparisons). In addition,

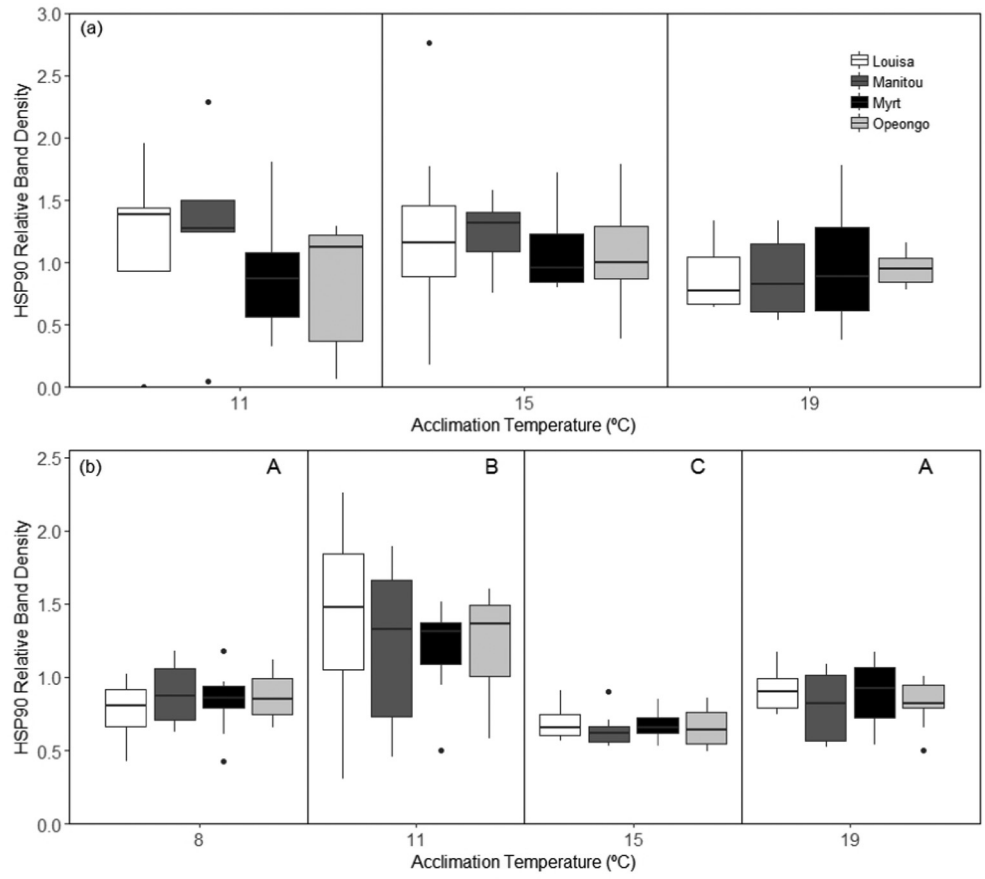

Fig. 2. Basal HSP90 (a) and heat-shocked HSP90 (b) levels of four lake trout populations acclimated to four temperatures [8 °C, 11 °C, 15 °C, and 19 °C]. Levels are expressed relative to a control (heat-shocked sample). The boxes represent the first and third quartiles, the horizontal lines represent the median, the whiskers represent fifth and ninety-fifth percentiles, and points outside of these whiskers represent outliers. All outliers were retained in the analysis. Significant differences among acclimation temperatures based on Dunn's test with Bonferroni correction for multiple comparisons ($p < 0.05$) are indicated by upper case letters. Basal HSP90 in cardiac tissue did not vary significantly with acclimation temperature or among populations ($p > 0.05$). Acclimation had a significant effect on cardiac HSP90 in heat-shocked fish ($p < 0.05$); however, population did not ($p > 0.05$). There are no 8 °C basal HSP90 data presented in panel (a) due to quality issues from poor standards on the western blots for these samples (see Results section).

HSP90 was significantly higher in 8 °C– and 19 °C-acclimated fish compared with 15 °C (Dunn's test with Bonferroni correction: $p < 0.05$ for both comparisons). Heat-induced HSP90 expression in cardiac tissue did not vary among lake trout populations (Fig. 2(b); $F_{3,144} = 0.42$, $p = 0.74$). As was the case with HSP70, there was no significant interaction between acclimation temperature and population (Fig. 2(b) $F_{9,144} = 0.62$, $p = 0.78$).

4. Discussion

4.1. HSP expression in Lake trout cardiac tissue

The patterns of lake trout cardiac HSP expression varied between HSP70 and HSP90. We did not observe inducible cardiac HSP70 expression in basal fish at any acclimation temperature. An acute heat shock to 23 °C induced HSP70 synthesis in cardiac tissue at all acclimation temperatures (Fig. 1). This was expected, as HSP70 is a commonly occurring heat-induced molecular chaperone (Feder and Hofmann, 1999). Cardiac HSP90 was detected in both basal and heat-shocked lake trout (Fig. 2). The expression of HSP90 in basal fish is not surprising, as HSP90 functions as a molecular chaperone in a several cell signalling pathways and supports the cytoskeleton (Iwama et al., 2004). Our data show that, similar to other salmonids, lake trout are capable of eliciting a HSR upon exposure to physiologically stressful temperatures.

4.2. Plasticity of the heat shock response

Cardiac HSP expression of lake trout populations in this study exhibited a plastic response to thermal acclimation. Induced HSP70 levels were similar across much of the acclimation temperature range (8 °C–15 °C), but declined significantly in 19 °C-acclimated fish. Thermal acclimation had no effect on basal cardiac HSP90 levels (Fig. 2), although there was an effect in heat-shocked fish (an increase at 11 °C; Fig. 2). The HSP90 antibody we used does not discriminate between inducible HSP90 and upregulated constitutive HSP90 isoforms, therefore, we compared HSP90 levels before and after heat shock and observed that the heat-induced synthesis of HSP90 in cardiac tissue was dependent on acclimation temperature (Fig. 3). For lake trout acclimated to 11 °C, which is within the optimum temperature range for this species (8 °C–12 °C; Christie and Regier, 1988), heat-shocked fish had higher cardiac HSP90 levels than basal fish (Fig. 3). In contrast, heat-shocked fish acclimated to 15 °C and 19 °C had lower cardiac HSP90 levels compared to basal fish (Fig. 3). The lower cardiac HSP70 and HSP90 observed at higher acclimation temperatures could be due to the relative magnitude of the heat shock experienced across acclimation temperatures. Thermal acclimation can alter properties of the HSR in fishes such that warm-acclimated fish exhibit a higher onset temperature (T_{on}), temperature of maximal induction (T_{peak}) and/or cessation temperature (T_{off}) of HSP expression compared to cold-acclimated fish (Dietz and Somero, 1992; Tomanek and Somero, 1999; but see Koban et al., 1987). Thermal acclimation to 15 °C and 19 °C might require a higher heat shock temperature to induce (T_{on}) and/or maximize (T_{off}) cardiac HSP expression in the lake trout populations examined in this study. Previous experiments have demonstrated that the thermal tolerance (CTM) of warm-acclimated lake trout is 2–3 °C higher than cold-acclimated fish over similar acclimation temperature ranges used in this study (McDermid et al., 2013; Kelly et al., 2014). A shift in the cardiac HSR with warm-acclimation could assist juvenile lake trout in coping with exposure to elevated temperatures, up to a thermal limit. However, in order to conclude definitively that warm-acclimation shifted the T_{on} of HSP synthesis, future experiments should expose lake trout to multiple heat shock temperature treatments to describe how thermal acclimation alters different properties of the HSR in this cold-adapted salmonid.

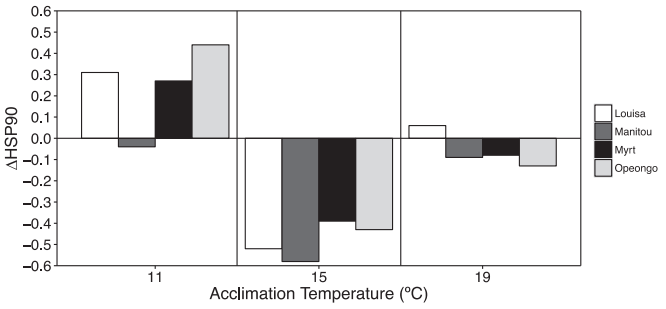

Fig. 3. The difference in mean cardiac HSP90 levels between basal and heat-shocked lake trout (HSP90 after heat shock to 23 °C – Basal HSP90 at acclimation temperature). Values used in calculations were least squares means from GLS model outputs.

The results of our study support previous research that suggests cardiac tissue is vulnerable to thermal stress in juvenile salmonids and the HSR (HSP70 and HSP90) induced to cope with this stress is plastic upon thermal acclimation. Similar to our results on lake trout, previous work on juvenile rainbow trout and brook trout reported basal cardiac HSP70 levels that were either barely detectable or completely absent, as well as significant induction of cardiac HSP70 and/or HSP90 after an acute heat shock (to 25 °C) (Rendell et al., 2006; Fowler et al., 2009; Stitt et al., 2014). These studies used a similar heat shock protocol to this study (3 °C·h^{-1} rate of increase from acclimation temperature to test temperature, 1 h heat shock, and up to 24 h recovery). Thermal acclimation had a significant effect on cardiac HSP70 in brook trout such that fish acclimated to temperatures within the optimum range for this species (12 °C and 16 °C) had higher cardiac HSP70 compared to fish acclimated to temperatures outside this range (8 °C and 20 °C; Stitt et al., 2014). This is similar to the effect of warm acclimation on cardiac HSP70 expression in lake trout reported here; however, due to a similar experimental design it cannot be ruled out that the decline in cardiac HSP70 in 20 °C-acclimated brook trout was not also due to an acclimation-induced shift in the T_{on} for the populations in that study.

4.3. Intraspecific variation

There was limited evidence of inter-population variation in the HSR (cardiac HSP70 and HSP90) among the four lake trout populations examined 24 h after heat shock at 23 °C. There was no effect of population on cardiac HSP90 and post-hoc pairwise comparisons failed to detect a difference in induced cardiac HSP70 among any of the populations despite a significant main effect of population in our model. Our data suggest that these populations have a similar ability to cope with thermal stress in cardiac tissue. This supports our previous work where we showed that the thermal tolerance of these four populations did not differ over the same acclimation temperature range (Kelly et al., 2014). However, body tissues can vary widely in HSP expression (Rendell et al., 2006; Stitt et al., 2014), thus, future investigations of other tissues and/or HSP families in lake trout could further elucidate the degree of variation the capacity of these populations to cope with thermal stress.

The results of our study add to the accumulation of information on local adaptation of cardiac physiology in salmonids exposed to elevated temperatures (Eliason et al., 2011; Chen et al., 2013; Anttila et al., 2014a, 2014b; Gradil et al., 2016). Previous research on intraspecific variation in the HSR among fish populations has yielded conflicting results. After a 1 h heat shock at 25 °C and 24 h recovery period, cardiac HSP70 varied significantly among brook trout strains consistent with their ancestral thermal history (Stitt et al., 2014). Sockeye salmon exposed to a thermal challenge exhibited variation between populations in cardiac *hsp90β* gene expression but not in *hsp90α* and *hsp30* gene expression (Anttila et al., 2014b). Similarly, redband trout populations from desert and montane climates exhibited local adaptation in gene expression for some *hsp* genes (*hsp70 and hsp47)* but not others (*hsp90*

and *hsp27*) (gill and liver, Narum et al., 2013). In contrast, Werner et al. (2006) reported considerable overlap in HSP70 and HSP90 expression (white muscle) in coastal and inland steelhead trout (*O. mykiss*) populations 6 h and 24 h after a 25 °C heat shock. A lack of difference in the magnitude of the response across lake trout populations, at least under our experimental conditions, might reflect the evolutionary history of the species, where genetic variation may have been constrained from repeated glaciations, post-colonization restrictions on local population sizes and finite ancestral populations in glacial refugia (Bernatchez and Wilson, 1998; Willi et al., 2006). It is also possible that, despite their geographic separation, the limited variation among the four populations might reflect the homogeneity of thermal habitats used by lake trout (Martin and Olver, 1980; Plumb and Blanchfield, 2009; Evans, 2007). As a consequence populations may have experienced similar selective pressures for thermal tolerance. Expansion of our study across a wider geographic range, and specifically targeting lakes known to differ in thermal properties, would allow these competing hypotheses to be tested. Furthermore, including multiple heat shock temperatures and recovery periods, as well as comparing protein versus mRNA levels, would improve our understanding of interpopulation variation in lake trout HSP expression by allowing for comparisons of different properties of the HSR (T_{on}, T_{off}, and T_{peak}).

The four lake trout populations we studied did not differ in their acclimation capacity of cardiac HSP expression (i.e., there was no significant interaction between acclimation temperature and population). A lack of variation among populations in acclimation capacity is consistent with previous work on critical thermal maxima (McDermid et al., 2013; Kelly et al., 2014) and metabolism (routine metabolic rate, maximum metabolic rate, and metabolic scope; Kelly et al., 2014) in these same populations. In contrast, studies of other species that have investigated intraspecific variation in the capacity for thermal acclimation of physiological traits often report significant variation among populations (Sylvestre et al., 2007; Seebacher et al., 2012; Stitt et al., 2014). The capacity for thermal acclimation plays a critical role in the ability of populations to endure environmental change (Stillman, 2003; Somero, 2010). The lack of variation in thermal acclimation capacity, as well as limited variation among populations for the cardiac HSR (this study) and other physiological traits (McDermid et al., 2013; Kelly et al., 2014) collectively suggest that lake trout populations could be similarly affected by climate-related temperature elevations across much of their geographic range.

4.4. Climate change implications

Cold-adapted fish populations are vulnerable to the predicted effects of climate change (Sharma et al., 2011; Lynch et al., 2016; Whitney et al., 2016). Climate-induced temperature elevations have been documented in lake ecosystems over the past few decades (Kraemer et al., 2015), and empirical models project that lake surface-water temperatures will increase significantly (to 25 °C – 30 °C for the lake trout populations in this study) over the next century (Sharma et al., 2007). Due to their restrictive habitat requirements (cold, well-oxygenated water generally < 12 °C; Evans, 2007), lake trout are largely confined to the hypolimnetic layer in lakes during summer months when lakes are thermally stratified (between spring and fall turnover) (Plumb and Blanchfield, 2009). Accordingly, their habitat is thermally extremely stable, and varies very little among lakes across the species range (Martin and Olver, 1980; Evans, 2007; Plumb and Blanchfield, 2009). Climate-induced elevations in water temperature will increase the duration and magnitude of summer thermal stratification and reduce the amount of optimal habitat (temperature and oxygen) for lake trout (Ficke et al., 2007; Herb et al., 2014; Guzzo and Blanchfield, 2016). In many lakes, this will force these cold-adapted fish to move into warmer water layers to avoid asphyxiation and to forage, which could have negative consequences on metabolism (Evans, 2007; Kelly et al., 2014), growth and reproduction (Plumb et al., 2014; Guzzo et al., 2017). Understanding their ability to cope with higher temperatures is important for the management of this species and is relevant to their long-term sustainability.

The reduced cardiac HSP expression we observed in warm-acclimated lake trout suggests that the induction of HSP expression might require a higher temperature in these fish. Cardiac tissue might adjust to physiologically stressful temperatures via thermal acclimation in a manner which reduces protein damage. Cardiac physiology plays a critical role in maintaining aerobic metabolic scope and defining the upper thermal tolerance limits in salmonids experiencing elevated temperatures (Farrell et al., 2009; Eliason et al., 2011), thus, this plasticity in cardiac function could be beneficial for populations that are exposed to thermal stress as climate-induced temperature elevations alter the thermal properties of freshwater habitats during the next century (Ficke et al., 2007; Somero, 2010).

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